84-2 - Unscheduled DNA Synthesis in Rat Hepatocytes

Reviewed by: John H.S. Chen John H.S. Chen 4/30/87
Section I, Toxicology Branch (TS-769C)
Secondary reviewer: R.B. Jaeger
Section I, Toxicology Branch (TS-769C) RBJ 5/7/87

DATA EVALUATION REPORT

Study Type: DNA Damage

TOX. CHEM. No.: 298C

Accession No.: 7E3489

MRID No.:

Test Material: CGA 154281 Technical (93.9% Purity)

Study Number(s): 860177

Sponsor: CIBA-GEIGY Corp.

Test Facility: Experimental Pathology, CIBA-GEIGY Limited, Basal, Switzerland

Title of Report: Autoradiographic DNA Repair Test on Rat Hepatocytes

Author(s): E. Puri, I. Hunziker, K. Mennle

Report Issued: October 24, 1986

Conclusions:

CGA 154281 Technical did cause DNA damage inducible repair in the rat hepatocytes at the concentrations tested (3 through 20 ug/ml).

Concentrations tested: 0.12, 0.6, 3, and 15 ug/ml in the first trial and 0.125, 0.25, 0.5, 1, 5, 10, 15, and 20 ug/ml in the second trial.

Classification of Data: Acceptable

Title of Report: Autoradiographic DNA Repair Test on Rat Hepatocytes with CGA 154281 Technical

Procedure:

Freshly idolated hepatocytes from a male rat (Tif, RAIF-SPF, Weight: 240 g) were cultivated in William's medium E containing 10 percent fetal bovine serum. The procedure used for this study is outlined below:

A series of compartments in petri plates containing Thermanox coverslips were seeded with 4 X 10^5 cells per compartment (density 10^5 cells/ml; 4 mml/compartment). The cells were allowed to attach to the coverslips during an attachment period of 1.5 - 2 hours. They were then washed and cultivated overnight in fresh medium. On the following morning, the test compound was dissolved in DMSO and the preselected concentrations of the test compound (1st Experiment: 0.12, 0.6, 3, and 15 ug/ml; 2nd Experiment: 0.125, 0.25, 0.5, 1.5, 10, 15, and 20 ug/ml) were prepared.

A volume of 10 ul from each test concentration was added to one compartment. Immediately after addition of the test compound, ^{3}H-thymidine (4 uCi/ml) was also added to the compartment. The culture plates were incubated for 5 hours at 37 C. After the incubation, the cells were washed twice with balanced salt solution, and fixed with ethanol/acetic acid (3/1, v/v). The coverslips were mounted on microscope slides and prepared for autoradiography. After 6 days of exposure period, the autoradiographs were stained with hematoxylin-eosine.

From each of the treated groups and from the positive and the negative controls, 150 nuclei in altogether three slides (50 cells/slide) were scored. The test compound was reported positive when the mean number of net silver grains per nucleus in relation to the solvent control was more than doubled at any concentration.

Results:

(1) Preliminary Toxicity Test

Treatment		Adehesion and Condtion of the Cells	% Viable Cells
DMSO (1%)		+	94
CGA 154281	15.7 ug/ml	±	71
"	31.5 "	±	NE
"	62.5 "	±	NE
"	125 "	-	NE
"	250 "	-	NE
"	500 "	-	NE
"	1000 "	-	NE

+ = Adequate number of adhered cells, which were in good condition; ± = Adequate number of adhered cells, which were in bad condition; - = Unadequate number or no adhered cells; NE = No evaluation (less than 25% of the cells were viable).

(2)

(1) Preliminary Toxicity Test - continued

Findings:

Based on the results obtained from this study, the highest usable concentration for this DNA-Repair assay was found to be at the concentration of 15 ug/ml CGA 154281 Technical.

(2) Summary of Unscheduled DNA Synthesis Data

Treatment	No. of Coverslips	No. of Nuclei Counted	Mean No. of Silver Grain/ Nucleus	Mean No. of Silver Grain/ Cytoplasm	Mean No. of Net Silver Grain/Nucleus
1st Trial:					
Medium	3	150	1.33	1.26	0.07
DMSO	3	150	1.92	1.78	0.14
Positive Control (4-ABP, 50 uM)	3	150	13.03	5.00	8.03*
CGA 154281					
0.12 ug/ml	3	150	1.70	1.58	0.12
0.60 "	3	150	1.63	1.73	-0.10
3.0 "	3	150	2.05	1.61	0.44*
15.0 "	3	150	2.83	1.97	0.86*
2nd Trial:					
Medium	3	150	1.44	0.96	0.48
DMSO	3	150	1.42	1.20	0.22
Positive Control (4-ABP, 50 uM)	3	150	13.29	3.30	9.99*
CGA 154281					
0.13 ug/ml	3	150	1.67	1.69	-0.02
0.25 "	3	150	1.88	1.28	0.60*
0.50 "	3	150	1.83	1.55	0.28
1.0 "	3	150	2.07	2.00	0.07
5.0 "	3	150	2.91	1.68	1.23*
10.0 "	3	150	3.23	1.90	1.33*
15.0 "	3	150	3.03	1.81	1.22*
20.0 "	3	150	3.34	1.88	1.46*

* Positive Response: The number of net silver grain per nucleus in relation to the concurrent control value was doubled.

Findings:

1. The labeling in the solvent control was found to be within the normal range of net silver grain count (0.14 to 0.22).

2. In the first DNA-Repair assay, comparison of the mean number of net silver grains per nucleus in the vehicle control (0.14) and after treatment with 3 and 5 ug/ml of CGA 154281 revealed a dose-related increase in this study. In the second trial, a dose-related increase in the mean number of net silver grains was also found in the treated rat

(2) Summary of Unscheduled DNA Synthesis Data - continued

Findings:

hepatocytes (5, 10, 15, and 20 ug/ml) when compared to that of the solvent control.

3. The positive control compound (4-Aminobiphenyl) had an expected high net silver grain count per nucleus in the range of 8.03 to 9.99.

Evaluation:

Under the test conditions reported, the test compound, CGA 154281 Technical induced a significant increase in the nuclear labeling of rat hepatocytes and also exhibited a dose-response relationship at the dosage levels tested (3 through 20 ug/ml). Therefore, CGA 154281 Technical is considered active in the unscheduled DNA synthesis in rat hepatocytes. This study is acceptable.